REMARKS

Reconsideration of the rejections set forth in the Office action mailed December 14, 2002 is respectfully requested. Claims 1-8 are currently under examination. Claims 2 and 4 are cancelled per this amendment. Claims 9-16 have been withdrawn following restriction.

I. Amendments

Claim 1 has been amended to recite that the heteroduplexes formed in step (b) are treated with an exonuclease whose substrate is double stranded DNA (as recited in original claim 2, now cancelled). The claim further recites that the heteroduplexes have a region at one terminus in which both strands are in single stranded form, and that, as a result, the exonuclease converts substantially every perfectly matched duplex to single stranded DNA and substantially every mismatched duplex to partially double stranded DNA.

Support is found, for example, in Figure 1C, which shows that regions **192** and **154** of amplicons **210** and **210'** are non-hybridizing and thus in single stranded form. Conversion of matched duplexes to single stranded DNA, and mismatched duplexes to partially double stranded DNA, is described, for example, at page 10, lines 25-30, and illustrated in Figure 1C.

Claim 1 further recites that the partially double stranded DNA is then extended to form double stranded heteroduplexes, followed by amplification, as described at page 10, lines 30-32 and page 11, line 1, and illustrated in Figure 1C.

Claims 3 and 4 have been combined and amended to recite that the sequence of the fourth primer binding site is different from that of any of the other primer binding sites, as disclosed at page 9, lines 9-10 of the specification. Claims 3 and 5 are amended to correct dependency.

No new matter is added by any of the amendments.

II. Rejections under 35 U.S.C. §102(e)

Claims 1-2 and 7-8 were rejected under 35 U.S.C. §102(e) as being anticipated by Stefano (U.S. Patent No. 6, 297,010). This rejection is respectfully traversed for the following reasons.

The standard for lack of novelty, that is, for anticipation, is one of strict identity. *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F2d 1367, 231 USPQ 81, 90 (Fed. Cir. 1986); *In re Donohue*, 766 F2d 531, 226 USPQ 619, 621 (Fed. Cir. 1985). To anticipate a claim for a patent, a single prior source must contain all its essential elements.

A. The Invention

The applicant's invention, as embodied in claim 1, provides a method of identifying polymorphic DNA sequences in a test DNA population, the method comprising the steps of:

(a) providing a reference DNA population;

(b) forming a population of heteroduplexes from single stranded DNA of the reference DNA population and single stranded DNA of the test DNA population, said heteroduplexes having at one terminus a region in which both strands are in single stranded form;

(c) treating said heteroduplexes with an exonuclease whose substrate is duplex DNA, such that said exonuclease converts substantially every perfectly matched duplex to single stranded DNA and substantially every mismatched duplex to partially double stranded DNA;

(d) extending said partially double stranded DNA to form double stranded heteroduplexes;

(e) amplifying the double stranded heteroduplexes to form a population of amplicons; and

(f) determining the nucleotide sequence of a portion of each amplicon so that polymorphic DNA sequences of the test DNA population are identified.

B. The Cited Art

Stefano teaches a method of identifying polymorphic DNA sequences in a test DNA population. A key feature of the method is protection of the area of the duplex surrounding the mismatched polymorphic segments, using an agent which binds specifically to the mismatched region, as shown in Figure 1A, and described, for example, at column 3, lines 36-41. The complex formed is then "contacted with an agent that removes unprotected base pairs, such as a 3'→5' exonuclease, to form a single-stranded region terminating at a position of the agent" (column 3, lines 41-44, also illustrated in Fig. 1A). As shown in Figure 1A, this treatment renders mismatched heteroduplexes single stranded, except at the protected area surrounding the mismatch. The treatment also destroys "unbound DNA", that is, matched heteroduplexes to which the protecting agent is not bound.

It can be seen from Figure 1A of the reference that, without the protecting agent, the treatment would substantially destroy mismatched duplexes as well, even if a nuclease requiring a double stranded substrate is used.

The reference does not show or suggest providing a test/reference DNA heteroduplex

"having at one terminus a region in which both strands are in single stranded form", such that the nuclease "converts substantially every perfectly matched duplex to single stranded DNA and substantially every mismatched duplex to partially double stranded DNA".

Since the reference does not disclose all of the elements set out above in claims 1-2 and 7-8, the claims cannot be anticipated by this reference under 35 U.S.C. §102(b). In view of this, the applicant respectfully requests the Examiner to withdraw the rejection under 35 U.S.C. §102(b).

Nor would the claimed invention have been obvious over Stefano per se. As shown in Figure 1A, the method of Stefano, where duplexes are treated with an "agent that removes unprotected base pairs, such as a 3'→5' exonuclease", renders mismatched heteroduplexes single stranded, except at the protected area surrounding the mismatch. The treatment also destroys "unbound DNA", that is, matched heteroduplexes to which the protecting agent is not bound. There would be no reason to employ in this method a duplex having a terminus at which both strands are in single stranded form, as claimed by the applicant, as this could interfere with the action of the "agent" (e.g. the exonuclease), thus defeating the purpose of the method.

It can also be seen from Figure 1A of the reference that, without the protecting agent, the method of Stefano would substantially destroy mismatched duplexes as well as matched duplexes. The applicant's method, by employing heteroduplexes having at one terminus a region in which both strands are in single stranded form, renders this terminus not susceptible to digestion by the exonuclease. The double stranded structure of the duplex is thus preserved, up to the mismatch point (as shown at **212** on the left side of Figure 1C). The method therefore does not require the area of the duplex surrounding the mismatch to be "protected" by a binding agent, as in Stefano.

III. Rejections under 35 U.S.C. §103(a)

Claims 3-4 were rejected under 35 U.S.C. §103(a) as being unpatentable over Stefano, cited above, in view of Weghorst *et al.* (U.S. Patent No. 6,080,544). The rejections are respectfully traversed in light of the following remarks.

A. The Invention

Claim 3 includes all the limitations of claim 1, and further provides that members of the reference DNA population of claim 1 are provided in a first cloning vector, and members of the

test DNA population of claim 1 are provided in a second cloning vector. The first cloning vector has a first primer binding site, a second primer binding site, a third primer binding site, and a cloning site disposed between the second and third primer binding sites. The second cloning vector has a fourth primer binding site, a fifth primer binding site, and a cloning site disposed between the fourth primer binding site and the fifth primer binding site, where the fifth primer binding site has a nucleotide sequence identical to the third primer binding site, and the sequence of the fourth primer binding site is different from that of any of the other primer binding sites.

B. The Cited Art

Stefano is discussed above.

Weghorst *et al.* discloses a method for mismatch detection in heteroduplexes in which the mismatched sites are chemically modified, e.g. by a single strand specific reagent, and the chemically modified sites are detected, e.g. by the use of antibodies (column 2, lines 53-61).

Examples 1-2 in Weghorst describe the use of cloning vectors for preparing test and reference DNA populations, as pointed out by the Examiner. As described at column 9, lines 55-57 and column 15, lines 42-45, the same vector was apparently used for both populations. Therefore, there is no indication that first and second cloning vectors, where one of the primer binding sites in the second vector was "different from that of any of the other primer binding sites", as claimed, were used.

C. Analysis

Even if one employed the pBluescript KSII+ cloning vectors of Weghorst *et al.* in preparing reference and test DNA populations in the method of Stefano, the resulting method would not include or suggest all of the features of applicant's claims 1 and 3. Neither reference, alone or in combination, suggests a method in which a test/reference DNA heteroduplex has, at one terminus, a region in which both strands are in single stranded form, such that an exonuclease "converts substantially every perfectly matched duplex to single stranded DNA and substantially every mismatched duplex to partially double stranded DNA", as provided in the parent claim.

In addition, neither reference suggests the use of first and second cloning vectors as recited in dependent claim 3, where one of the primer binding sites in the second vector is "different from that of any of the other primer binding sites".

In view of the foregoing, the applicant respectfully requests the Examiner to withdraw the rejection of claim 3 under 35 U.S.C. §103(a).

IV. Further Rejections under 35 U.S.C. §103(a)

Claims 5-6 were rejected under 35 U.S.C. §103(a) as being unpatentable over Stefano, cited above, in view of Weghorst *et al.*, cited above, and further in view of Nikiforov *et al.* (*Nucleic Acids Research* **22**(20):4167-75 (1994). The rejections are respectfully traversed in light of the following remarks.

A. The Invention

Dependent claim 5 includes all the limitations of claims 1 and 3, and further provides that the single stranded reference DNA is generated by amplifying the reference population by PCR using a nuclease-resistant primer specific for the first primer binding site and a primer specific for the third primer binding site, to form an amplicon having a single strand with a nuclease-resistant 5' end, and digesting the amplicon with a 5'→3' exonuclease. Dependent claim 6 further provides that the single stranded test DNA is generated by amplifying the test population by PCR using a nuclease-resistant primer specific for the fourth primer binding site, and a primer specific for the fifth primer binding site, to form an amplicon having a single strand with a nuclease-resistant 5' end, and digesting the amplicon with a 5'→3' exonuclease.

B. The Cited Art

Stefano and Weghorst *et al.* are discussed above.

Nikiforov *et al.* discloses a "primer guided" method of detecting SNPs, in which a primer is hybridized adjacent the SNP and extended, and the nature of the extended base is determined (Abstract; also page 4168, first full paragraph).

The reference discloses that nuclease resistant groups were used to protect the 5' end of one strand of a duplex from nuclease digestion, in the preparation of single-stranded PCR fragments (page 4169, second full paragraph).

C. Analysis

Even if one employed the cloning vectors of Weghorst *et al.* in preparing reference and test DNA populations in the method of Stefano, and then used the method described in Nikiforov *et al.* to prepare single stranded DNAs from these populations, the resulting method would not include or suggest all of the features of applicant's claim 1 and dependent claims 3, 5, and 6. None of the references, alone or in combination, suggests a method in which a test/reference DNA heteroduplex is treated with an exonuclease, wherein "the heteroduplex has, at one

terminus, a region in which both strands are in single stranded form, such that said exonuclease converts substantially every perfectly matched duplex to single stranded DNA and substantially every mismatched duplex to partially double stranded DNA", as provided in the parent claim.

In view of the foregoing, the applicant respectfully requests the Examiner to withdraw the rejection under 35 U.S.C. §103(a).

V. Conclusion

In view of the foregoing, the applicant submits that the claims now pending are now in condition for allowance. A Notice of Allowance is, therefore, respectfully requested.

No further fees are believed due with this communication. However, the Commissioner is hereby authorized and requested to charge any deficiency in fees herein to Deposit Account No. 50-2207.

If in the opinion of the Examiner a further telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 838-4403.

Respectfully submitted,

Date: Mar 13, 2003

LeeAnn Gorthey
Registration No. 37,337

Correspondence Address:
PAYOR NUMBER 22918
PHONE: (650) 838-4403
FAX: (650) 838-4350

Amendments to claims filed March 14, 2003
U.S. Serial No. 09/786,254

1. (Amended) A method of identifying polymorphic DNA sequences in a test DNA population, the method comprising the steps of:

(a) providing a reference DNA population;

(b) forming a population of heteroduplexes from single stranded DNA of the reference DNA population and [from] single stranded DNA of the test DNA population, said heteroduplexes having at one terminus a region in which both strands are in single stranded form;

(c) [isolating mismatched heteroduplexes of the population by digesting a single stranded DNA of] treating said heteroduplexes with an exonuclease whose substrate is duplex DNA, such that said exonuclease converts substantially every perfectly matched duplex to single stranded DNA and substantially every mismatched duplex to partially double stranded DNA;

(d) extending said partially double stranded DNA to form double stranded heteroduplexes;

[(d)] (e) amplifying the [isolated mismatched heteroduplexes] double stranded heteroduplexes to form a population of amplicons; and

[(e)] (f) determining the nucleotide sequence of a portion of each amplicon so that polymorphic DNA sequences of the test DNA population are identified.

3. (Amended) The method of claim [2]1, wherein [said steps of providing said reference DNA population and said test DNA population include providing] members of said reference DNA population are provided in a first cloning vector, and [providing] members of said test DNA population are provided in a second cloning vector, wherein

the first cloning vector [having] has a first primer binding site, a second primer binding site, a third primer binding site, and a cloning site disposed between the second and third primer binding sites,

and the second cloning vector has a fourth primer binding site, a fifth primer binding site, and a cloning site disposed between the fourth primer binding site and the fifth primer binding site, wherein the fifth primer binding site has a nucleotide sequence identical to said third primer binding site, and the sequence of the fourth primer binding site is different from that of any of the other primer binding sites.

5. (Amended) The method of claim [4] 3, wherein said single stranded DNA of said reference DNA population is generated by amplifying said members of said reference DNA population in a polymerase chain reaction, using a nuclease-resistant primer specific for said first primer binding site and a primer specific for said third primer binding site, to form an amplicon having a single strand with a nuclease-resistant 5' end, and digesting the amplicon with a 5'→3' exonuclease.